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(54) Esters of boron analogues of amino acids.

(57) Aminoborane esters of formula $R_1R_2R_3NBH_2COOR_4$, wherein R_4 is C_1-C_8 alkyl, C_1-C_8 haloalkyl, phenyl, benzyl or cholesteryl and

(a) each of R_1, R_2, and R_3, which may be the same or different, is hydrogen, C_1-C_8 alkyl, C_1-C_8 haloalkyl, phenyl or benzyl with the proviso that at least one of R_1, R_2, and R_3 is hydrogen, or

(b) each of R_1, R_2, and R_3 is methyl with the proviso that R_4 is also methyl are useful in the lowering of serum cholesterol or triglyceride levels. These esters and related compounds can be prepared by esterifying the amine carboxyborane, preferably using a chloroformate in solution in the presence of a trialkylamine.

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DESCRIPTION

TITLE: ESTERS OF BORON ANALOGUES OF AMINO ACIDS

THIS INVENTION relates to boron analogues of amino acids, to methods of forming esters of boron analogues of amino acids and to the use of boron analogues of amino acids as a biologically active material for antitumour or hypolipidemic activity.

Boron analogues of amino acids are broadly known in the art. The antihyperlipidemic activity of amino cyanoboranes is discussed in Antihyperlipidemic Activity of Amino Cyanoboranes, Amino Carboxyboranes and Related Compounds, Journal of Pharmaceutical Sciences, Vol. 70, No. 3. March 1981.

US Patents related to boron analogues of amino acids include Nos. 4,209,510, 4,312,989 and 4,368,194.

This invention provides an amine borane ester compound of general formula I:

$$R_1R_2R_3NBH_2COOR_4$$

wherein R_4 is C_1-C_8 alkyl, C_1-C_8 haloalkyl, phenyl, benzyl or cholesteryl and

(a) each of R_1, R_2, and R_3, which may be the same or different, is hydrogen, C_1-C_8 alkyl, C_1-C_8 haloalkyl, phenyl or benzyl with the proviso that at least one of R_1, R_2, and R_3 is hydrogen, or

(b) each of R_1, R_2, and R_3 is methyl with the proviso that R_4 is also methyl.

The alkyl groups can be straight or branched chains. Representative alkyl groups include methyl, ethyl, propyl and n-butyl. In general, the preferred moieties are methyl and ethyl groups because these moieties have minimal steric hindrance during synthesis. In the new compounds it is preferred that R_1 is hydrogen

and R_2 and R_3 are both methyl while R_4 can be methyl or ethyl or possibly haloalkyl, phenyl, benzyl or cholesteryl.

Compounds of this invention as well as related compounds can be prepared by condensing the corresponding acids and alcohols with dicyclohexylcarbodiimide (DCC) at room temperature in dichloromethane and using a method disclosed hereinafter, using chloroformates, both methods forming part of the present invention.

The formation of amino carboxyborane esters as described above has provided a technique for making sufficient quantities of material for testing. However, the reaction times for the production of the compounds by condensation of the corresponding acids and alcohols with DCC at room temperature in CH_2Cl_2 are very long. The reaction generally gives good to moderate yields but is time consuming, i.e., up to a week or more. Moreover, the esters contain dicyclohexylurea as a by-product which must be removed. Separation of the desired product by fractional crystallisation and solvent extraction is tedious and difficult owing to the similar solubility characteristics of the desired ester and the by-products.

An alternative synthesis technique for making the ester is the treatment of trialkylaminecarboxyborane with a tetrafluoroborate compound, as disclosed in US Patent 4,368,194, was effective for compounds where the initial carboxyborane had a triamine substituent. It does not work with carboxyboranes of diamines, monoamine or ammonia since the hydrogens on the boron in the borane hydrolyse.

A new and more efficient synthesis with a more general application is disclosed in this Application. The new synthesis can be used to produce esters regardless of the presence or absence of hydrogen atoms on the nitrogen of the amine carboxyborane. In general, the desired esters are formed by the reaction of mixed carboxylic-carbonic

anhydrides under mild conditions. In the improved synthesis, mixing equimolar amounts of amine-carboxyborane, alkylchloroformate, and trialkylamine in methylene chloride at reduced temperatures i.e., -10 to +10°C produces a rapid decarboxylation to give the desired ester product.

As an example, 0.01 mole of alkylchloroformate and 0.001 mole of dimethylaminopyridine were added to a solution of 0.01 mole of amine-carboxyborane and 0.011 mole of triethylamine in 100 ml of methylene chloride. The mixture was maintained at 0°C for one hour with constant stirring. The reaction proceeded smoothly with the evolution of carbon dioxide. After one hour, the solution was given two washings with 20 ml of water and dried using magnesium sulphate. The resulting material was concentrated to pure ester.

Using various starting materials, esters of the basic configuration $(R)_3N.BH_2COOR'$ were made using various starting compounds. Esters were formed where R was methyl or ethyl. The number of organic moieties attached to the amino nitrogen was varied from 1 to 3.

Esters were formed where R' was methyl, ethyl, cholesteryl, toluene, benzene, bromoethyl and chloroethyl.

This method allows a rapid production at high yields of a wide variety of ester materials.

A further aspect of the present invention provides a pharmaceutical composition for lowering of serum cholesterol or triglyceride level which comprises a therapeutically effective amount of at least one of the amine-carboxyborane esters of formula I and a pharmaceutically acceptable carrier. The invention also extends to the esters of formula I and the compositions containing them for use in a method of surgery or therapy on the human or animal body or in a method of diagnosis practised on the human or animal body.

The following Examples are given to illustrate the invention.

EXAMPLE 1

Trimethylamine-carbethoxyborane $(CH_3)_3N.BH_2COOC_2H_5$, was prepared by dehydrating a solution of $(CH_3)_3N.BH_2COOH$ and absolute ethanol with DCC at room temperature for 6 days. This procedure resulted in a 45% yield. The relatively high volatility and solubility in water of this sweet-smelling ester probably contributed to its low yield in this procedure.

EXAMPLE 2

Trimethylamine-carbomethoxyborane $(CH_3)_3N.BH_2COOCH_3$ was prepared with an 82% yield by condensing $(CH_3)_3N.BH_2COOH$ and CH_3OH with DCC at room temperature for one week; extension of the reaction period to two weeks led to an increase in the yield to 98%.

EXAMPLE 3

Dimethylamine-carbomethoxyborane $(CH_3)_3NH.BH_2COOCH_3$, was prepared in 67% yield by an amino-exchange reaction of $(CH_3)_3N.BH_2COOCH_3$ with an 8-fold excess (by weight) of $(CH_3)_2NH$ in a glass pressure reaction vessel for 2 weeks at room temperature. The 8% unreacted starting ester in the product mixture was removed by washing with H_20 and vacuum pumping. The ester linkages in the starting material and product were not cleaved by the excess amine. As an alternative, condensing $(CH_3)_2NH.BH_2COOH$ and CH_3OH with DCC at room temperature for 4 days could be performed. This reaction procedure gave a very low yield of 8%.

EXAMPLE 4

Methylamine-carbomethoxyborane, $CH_3NH_2.BH_2COOCH_3$ was prepared by condensing $CH_3NH_2.BH_2COOH$ and CH_3OH with DCC at room temperature for 6 days. This reaction had a 21% yield.

EXAMPLE 5

Trimethylamine-(carbo-2-chloroethoxy)borane, $(CH_3)_3N.BH_2COOCH_2CH_2Cl_{(s)}$, was prepared in a manner similar to the preparation of compound 4 by condensing $(CH_3)_3N.BH_2COOH$ and $HOCH_2CH_2Cl$ with DCC at room temperature for 1 week. This reaction yielded 61%.

EXAMPLE 6

Ammonia-carbomethoxyborane, $H_3N.BH_2COOCH_3$, was prepared by an amine-exchange reaction carried out in a stainless steel pressure vessel between $(CH_3)_3N.BH_2COOCH_3$ and excess liquid NH_3 at room temperature for 2 weeks.

EXAMPLE 7

Trimethylamine-(carbotrimethylsiloxy)borane, $(CH_3)_3N.BH_2COOSi(CH_3)_3$, was prepared by a procedure involving lithiation of $(CH_3)_3N.BH_2COOH$ with $n\text{-}C_4H_9Li$ under dry N_2 in ether and subsequent reaction of the lithium salt (not isolated) with $(CH_3)_3SiCl$ at ambient temperature for 16 hours. Work-up and vacuum distillation afforded 58% of the silyl ester as a clear, moisture-sensitive liquid that solidified on standing.

All of these compounds were characterised by elemental analysis and IR, H NMR and B NMR spectroscopy. Physical and spectral data of the esters are given in

Table I. IR spectra exhibited characteristic B-H and C=O absorptions; H and B NMR spectral data were consistent with the structures shown for the compounds. IR spectra were recorded on a Perkin-Elmer 297 spectrometer. Solid samples were prepared as KBr disks, as Nujol mulls between NaCl disks, or as solutions in suitable solvents; oils were recorded neat. Proton and boron NMR spectra were obtained on Varian EM 360A and JEOL FX 90Q spectrometers, respectively. Elemental analyses were performed by Galbraith Laboratories Inc., Knoxville, TN. or Schwarzkopf Microanalytical Laboratory Inc., Woodside, NY.

TABLE 1

compd	ester	bp/mp °C	yield %	shifts ppm[a]	J_{B-11}.Hz
1	$(CH_3)_3N.BH_2COOC_2H_5$	45-47	34-41	-9.17 (t)	98
2	$(CH_3)_3N.BH_2COOCH_3$	90-92	82-98	-9.09 (t)	99
3	$(CH_3)_2NH.BH_2COOCH_3$	52-53	67	-12.57 (t)	95
4	$CH_3NH_2BH_2COOCH_3$	56-57	21	-16.22 (t)	98
5	$(CH_3)_3N.BH_2COOCH_2CH_2Cl$		61	-8.75	97
6	$H_3N.BH_2COOCH_3$	92-93	49	-20.45 (t)	94
7	$(CH_3)_3N.BH_2COOSi(CH_3)_3$	60 (0.2 torr)	58		

[a] $(C_2H_5)_2O.BF_3$ was used as an external standard; all chemical shifts reported here were (negative) upfield from the standard.

SERUM LIPID SCREENING

CF_1 male mice (30 g) were fed rodent laboratory food with water ad libitum during the experiment. The compounds of this invention were suspended in 1% carboxymethylcellulose-water and homogenized. The doses

were calculated on the weekly weights of the mice. Test compounds were administered at a rate of 20 mg/kg/day ip. On the 9th and 16th days, blood was collected by tail vein bleeding in alkali-free, non-heparinised microcapillary tubes and centrifuged 3 minutes to obtain the serum. Duplicate 30-ml samples of non-hemolysed serum were used to determine the serum cholesterol levels (milligram percent) by a modification of the Liebermann-Burchard reaction described in Ness et al, Clin, Chem, Acta Vol. 10, page 229 (1964). A separate group of mice were bled on day 14, and their serum triglyceride levels (milligram percent) were determined by using 25-ml samples.

The results of the serum testing are set out in Table 2. The values given are percent control. The percentage inhibition, the effectiveness of the compound, is determined by subtracting the control percentage from 100.

TABLE 2

Percentage Control of Cholesterol and Triglyceride

Compound	I.P. Dose	Serum Cholesterol Day 9	Serum Cholesterol Day 16	Serum Triglyceride Day 16
$MeNH_2BH_2COOMe$	8	69	--	--
Me_3NBH_2COOMe	8	79	58	23
Me_2NHBN_2COOMe	8	73	68	69

All of the compounds show a degree of control on both serum cholesterol and triglycerides. In particular trimethylaminecarbomethoxyborane showed an inhibition

effect of 77% on serum triglycerides. There is a significant increase in activity over the previously reported activity of the corresponding ethyl ester which showed an inhibition of only 43% at a dosage of 20 mg/kg as reported in J. Pharm. Sci. 70, 339, (1981).

CLAIMS

1. An amine borane ester compound of general formula I:

$R_1R_2R_3NBH_2COOR_4$

wherein R_4 is C_1-C_8 alkyl, C_1-C_8 haloalkyl, phenyl, benzyl or cholesteryl and

(a) each of R_1, R_2, and R_3, which may be the same or different is hydrogen, C_1-C_8 alkyl, C_1-C_8 haloalkyl, phenyl or benzyl with the proviso that at least one of R_1, R_2, and R_3 is hydrogen, or

(b) each of R_1, R_2, and R_3 is methyl with the proviso that R_4 is also methyl.

2. A compound according to claim 1 wherein R_1 is hydrogen and R_2 and R_3 are each methyl or ethyl.

3. A compound according to claim 1 or 2 wherein R_4 is methyl or ethyl.

4. A compound according to claim 1 or 2 wherein R_4 is haloalkyl.

5. A compound according to claim 1 or 2 wherein R_4 is cholesteryl.

6. A compound according to claim 1 or 2 wherein R_4 is phenyl.

7. A compound according to claim 1 or 2 wherein R_4 is benzyl.

8. A compound according to claim 1 wherein each of R_1, R_2, R_3 and R_4 is methyl.

9. A method of forming an amine borane ester comprising the steps of: mixing equimolar amounts of an amine-carboxyborane and an aryl- or alkylchloroformate and at least an equimolar amount of a trialkylamine in a compatible solvent; and isolating the amine carboxyborane ester from the reaction mixture.

10. A method according to claim 9 wherein a catalytic amount of dimethylaminopyridine is added to the reaction mixture.

11. A method according to claim 9 or 10 wherein the aryl- or alkylchloroformate has the formula ClCOOR wherein R is a C_1-C_8 alkyl, C_1-C_8 haloalkyl, phenyl, benzyl or cholesteryl group.

12. A method according to any one of claims 9 to 11 wherein the amine carboxyborane has the formula $R_1R_2R_3NBH_2COOH$ wherein each of R_1, R_2 and R_3 is hydrogen or a C_1-C_8 alkyl, C_1-C_8 haloalkyl, phenyl or benzyl group.

13. A method according to any one of claims 9 to 12 wherein the trialkylamine is trimethylamine or triethylamine.

14. A method according to any one of claims 9 to 13 wherein the solvent is methylene chloride.

15. A method according to any one of claims 9 to 14 wherein the reaction is carried out at a temperature of from $-10^{o}C$ to $+10^{o}C$.

16. A method of forming an amine borane ester, which comprises condensing an amine borane carboxylic acid with an alcohol in the presence of dicyclohexyl-carbodiimide.

17. A method according to claim 16 wherein said condensing occurs in excess alcohol as a solvent.

18. A method according to claim 16 wherein said condensing occurs in a solution of CH_2Cl_2.

19. A pharmaceutical composition for the lowering of serum cholesterol or triglyceride level, which comprises a therapeutically effective amount of a compound according to any one of claims 1 to 8 and a pharmaceutically acceptable carrier.

20. A compound according to any one of claims 1 to 8 or composition according to claim 19 for use in a method of treatment of the human or animal body by surgery or therapy or in a method of diagnosis practised on the human or animal body.

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EUROPEAN SEARCH REPORT

0181721
Application number

EP 85 30 7732

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
D,A	US-A- 312 989 (B.F. SPIELVOGEL) * Column 1, line 20 - column 2, line 14; column 14, table 1 *	1-20	C 07 F 5/02 A 61 K 31/69
A	EP-A-0 034 238 (DUKE UNIVERSITY) * Examples; claims *	1-20	
P,X	CHEMICAL ABSTRACTS, vol. 102, no. 3, 21st January 1985, page 701, no. 24676a, Columbus, Ohio, US; B.F. SPIELVOGEL et al.: "Boron analogs of amino acids. 4. Synthesis of glycine and N-methylated glycine ester analogs", & INORG. CHEM. 1984, 23(25), 4322-4 * Abstract *	1-20	
P,X	MOL. CRYST. LIQ. CRYST., vol. 128, 1985, pages 65-73, Gordon and Breach Science Publishers Inc. and OPA Ltd., US; H. HAKEMI et al.: "Investigation of mixtures of cholesteryl esters of boron analogues of amino acids with p-azoxyanisole" * Pages 66,67 *	1-20	C 07 F 5/00 A 61 K 31/00

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	31-01-1986	PAUWELS G.R.A.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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